H$_2$S Test Strip, Modified is devised for simultaneous detection of *Salmonella, Vibrio, Citrobacter* species and *Escherichia coli*.

## Formula :

The medium for detection of hydrogen sulphide producers is soaked on rolled filter paper.

## Direction :

Fill the bottle with water upto arrow level. Allow to soak the rolled filter paper strip and shake gently. Keep at room temperature (30°C) or preferably at 35-37°C for 16-48 hours. If required to further verify the presence of *E. coli*, it is recommended to add 5-10 drops of Kovac's Indole Reagent (R008) to the bottles, after incubation.

Note: Add few drops of some disinfectant (i.e. dettol, phenyl etc.) and discard the bottle. Preferable to use the autoclave wherever the facility is available.

## Principle and Interpretation :

It has been reported that human faecal contamination is one of the main causes of water-borne diseases. In 1993, WHO (2) therefore recommended regular testing of drinking water for thermotolerant coliforms and *Salmonella* species to ensure its complete absence. The frequent testing of drinking water in remote areas, as well as in developing countries, is rather difficult to achieve. Townsend, 1992 (3) has demonstrated the lack of correlation between coliform bacteria and the presence of *Salmonella* species in water,

particularly in the tropics and subtropics. In Western Australia 30% of all *Salmonella* isolations from water have occurred in the absence of indicator bacteria (4). Iveson and Fleay 1991 (5), found that 3% of tropical waters tested were contaminated with *Salmonellae* in the absence of *Escherichai coli*. They suggested that the origin of *Salmonellae* may be from faeces of birds and reptiles which did not contain coliform bacteria. The absence of *Escherichia coli* in *Salmonella* contaminated water is more often in the tropics. However, analysis of *Salmonella* using the culture methods is a four stage process involving pre-enrichment, selective enrichment, biochemical identification and confirmation by serological method. Thus, it is a very lengthy process which requires four days for completion. Therefore Manja's (1) method is most suitable for the detection of *Salmonella* species which uses H$_2$S Strip. Ferric salts in the medium is reduced by certain species of enteric organisms to produce H$_2$S. In presence of oxygen, some bacteria are able to split tryptophan into indole and alpha amino propionic acid. Indole reaction can be detected by adding p-dimethylaminobenzaldehyde indicated by formation of a red coloured ring.

## Quality Control:

### Appearance:

Yellowish brown coloured, filter paper strip containing medium.
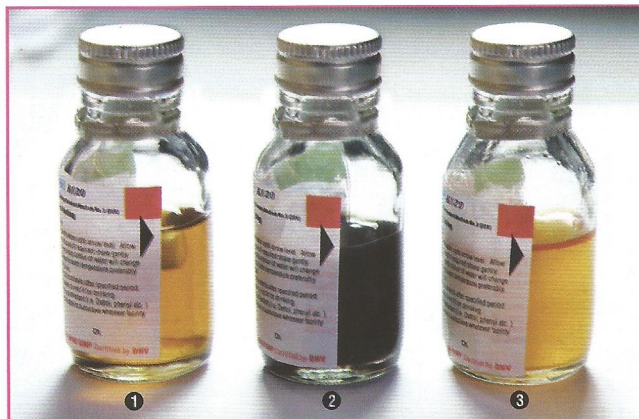


1. Control     2. *Salmonella* species / *C. freundii*     3. *E. coli*

## Colour and Clarity :

Amber coloured, clear solution obtained on addition of water.

## Cultural Response :

Cultural characteristics observed after an incubation at 35-37°C for 16-48 hours.

| Oraganism (ATCC) | Growth | Colour of Medium | H₂S production | Indole production |
|---|---|---|---|---|
| *E. coli* (25922) | luxuriant | yellow with haze | − | + |
| *S.* Typhimurium (23564) | luxuriant | black | + | − |
| *C. freundii* (8090) | luxuriant | black | + | − |
| *S.* Enteritidis *(13076)* | luxuriant | black | + | − |

Key : + = positive reaction    − = negative reaction

## Reference :

1. Manja K.S., Maurya M.S. and Rao K.M., 1982, A simple field test for the detection of faecal pollution in drinking water. Bulletin of the World Health Organization, 60:797-801.

2. WHO, 2006, Guidelines for drinking water quality, Vol. 1 Recommendations, 1st Addendum to 3rd edition.

3. Townsend S.A., 1992, The relationships between *Salmonellas* and faecal indicator bacteria concentrations in two pools in the Australia wet / dry tropics. Journal of Appl. Bacteriol. 73:182-188.

4. Peterson D.J., and Schorsch I., 1980, The microbiological surveillance of drinking water in Western Australia. WA Health Surveyor. 2 (June). 7-11.

5. Iveson J.B. and Fleay B.J., 1991, Serovars of *Salmonella* isolated from humans, animals, waters and effluents in natural and disturbed environments in Western Australia. Proceedings of the 14ᵗʰ Federal Convention, Australian Water and Wastewater, 2:435-441.

## Storage and Shelf-life :

Store below 30°C. It has shelf-life of 2 years.